Global lysine acetylation in *Escherichia coli* results from growth conditions that favor acetate fermentation

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Supplemental Table S2 and Figures S1-S4

Condition	Acetylated proteins	Acetylation sites (unique)
Xyl, 0.4%	542	**1608**
Xyl, 4%	811	**2824**
Glc, 0.4%	524	**1489**
Glc, 4%	814	**2949**

Table S2. Mass spectrometric analysis of acetyl-enriched peptide fractions. Overall, a total of 3,840 unique acetylation sites and a total of 978 acetylated proteins were identified by tandem mass spectrometry (MS/MS).

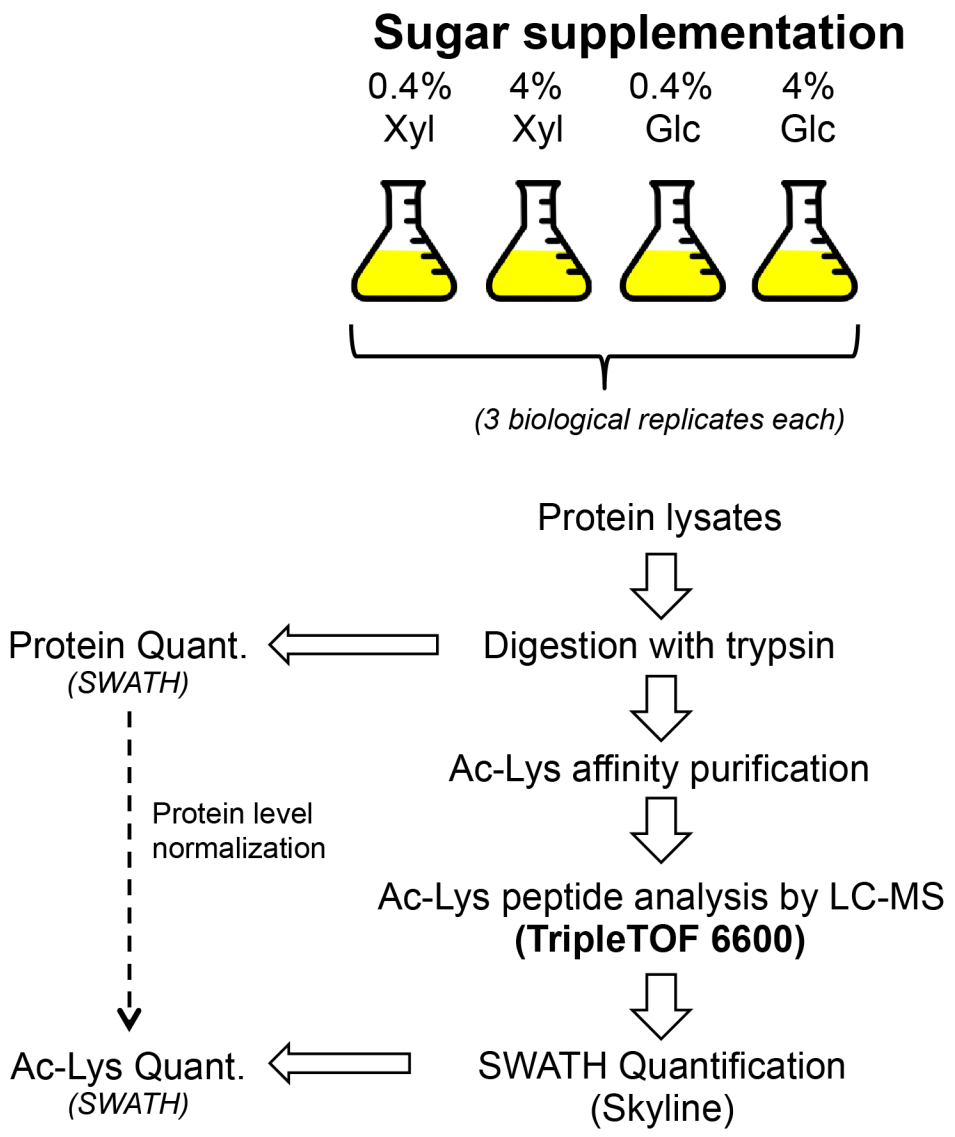

Figure S1. Mass spectrometric workflow to assess relative changes in acetylation site levels. Protein lysates are proteolytically digested and acetylated peptides are enriched by anti-acetyl affinity enrichment. Acetylated peptides are identified and quantified using modern proteomics technology (specifically data-independent acquisition, also referred to as SWATH).

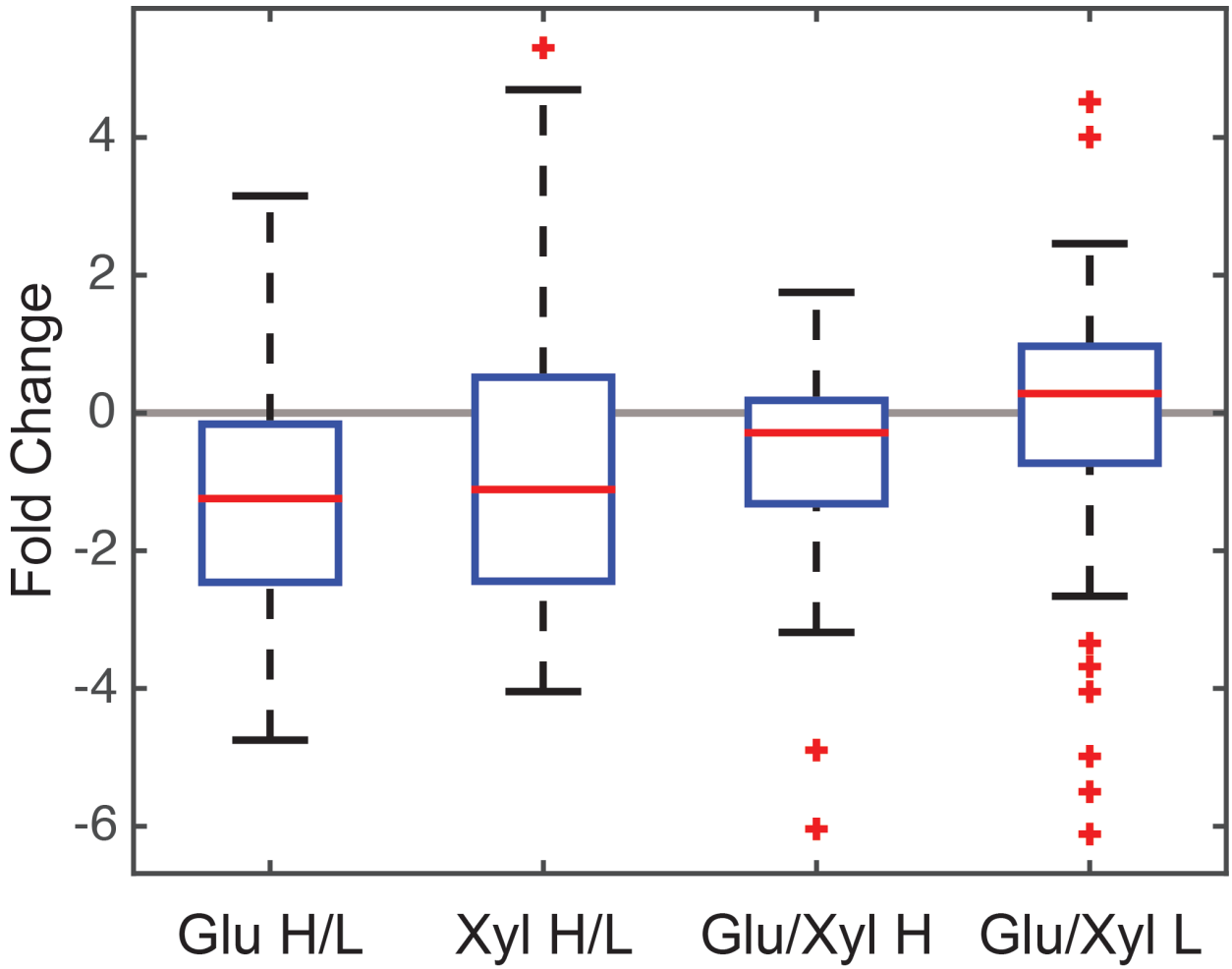

Figure S2. Differential protein expression under the four growth conditions. Cells were harvested after 12 hours of growth. For mass spectrometric analysis, *E. coli* cells were grown as described above in M9 minimal media supplemented with i) 0.4% glucose, ii) 4% glucose, iii) 0.4% xylose, or iv) 4% xylose. Isolated frozen bacterial pellets from each of the 4 growth conditions (3 biological replicates each) were lysed, followed by tryptic digestion of the protein lysate and mass spectrometric acquisition of all samples by data independent acquisition. Protein expression values are provided in **Table S1**. Abbreviations: Glu H/L (4% glucose versus 0.4% glucose); Xyl H/L (4% xylose versus 0.4% xylose); Glu/Xyl H (4% glucose versus 4% xylose); and Glu/Xyl L (0.4% glucose versus 0.4% xylose).

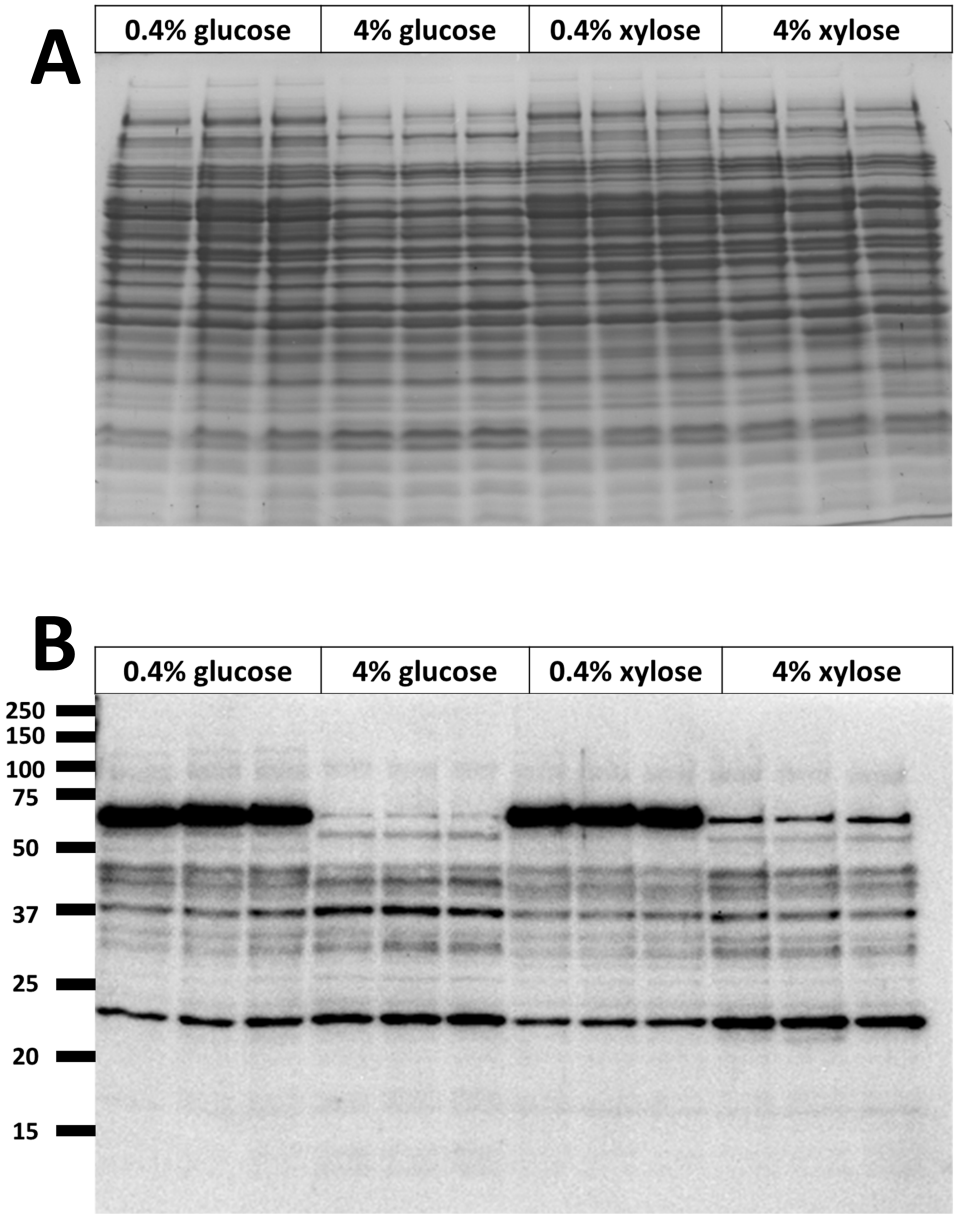

Figure S3. Relative changes in lysine acetylation under the four growth conditions as determined using anti-acetyllysine western blot. A. Coomassie blue-stained SDS-polyacrylamide gel and loading control for panel B. B. Antiacetyllysine Western blot of lysates from cells harvested from the indicated conditions at 12 h. The dark band is due to Acs (72.1

kDa), whose acetylation is catalyzed by the lysine acetyltransferase YfiQ (also known as Pka and PatZ) as opposed to non-enzymatic acetyl-phosphate-dependent acetylation (1). Transcription of *acs* decreases at higher sugar concentrations – due to catabolite repression.

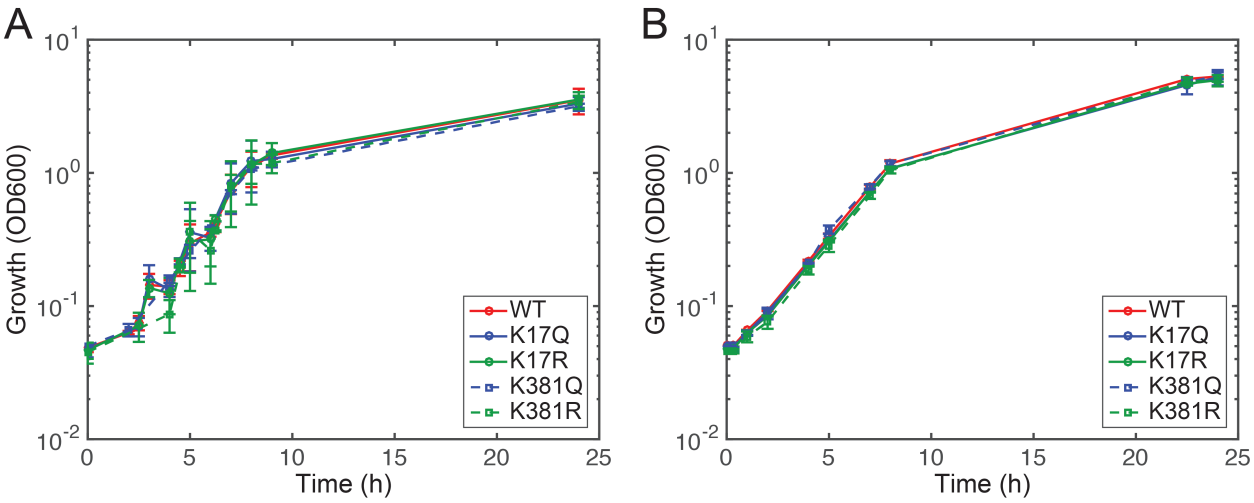

Figure S4. Comparison of growth for different *xylA* mutants during growth in (A) 0.4% xylose or (B) 4% xylose.

REFERENCES

1. Beatty CM, Browning DF, Busby SJ, Wolfe AJ. 2003. Cyclic AMP receptor protein-dependent activation of the Escherichia coli acsP2 promoter by a synergistic class III mechanism. J Bacteriol 185:5148-57.